CRISPR sgRNA Selection Site and Guide Efficacy Survey Site

C. elegans Gene Knockout Lab at UBC
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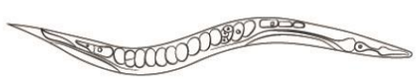
MOERMAN LAB

C. ELEGANS GENE KNOCKOUT FACILITY



CRISPR KNOCKOUT REQUESTS PLEASE EMAIL

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C. elegans
GUIDE SELECTION TOOL

genome.sfu.ca/crispr

C. elegans
GUIDE SURVEY & DATABASE

bit.ly/wormsurvey

Poster 600B!
Tonight!
7:30 – 9:00!
Vinci Au, Presenter

CRISPR sgRNA Selection Site

- Database of all pre-calculated NGG and NGA guides
- *C. elegans*-specific
- Simple interface similar to MMP web site
- Default filters
- Flexible optional filters and display options
- BED file available for display in IGV

Guide Efficacy Survey Site

- A database to improve sgRNA selection
- Contact information
- sgRNA sequence(s)
- Target gene
- sgRNA design source
- Construct/injection details
- Mutant selection details
- Did it work? Numbers, please!

CRISPR sgRNA Selection Site

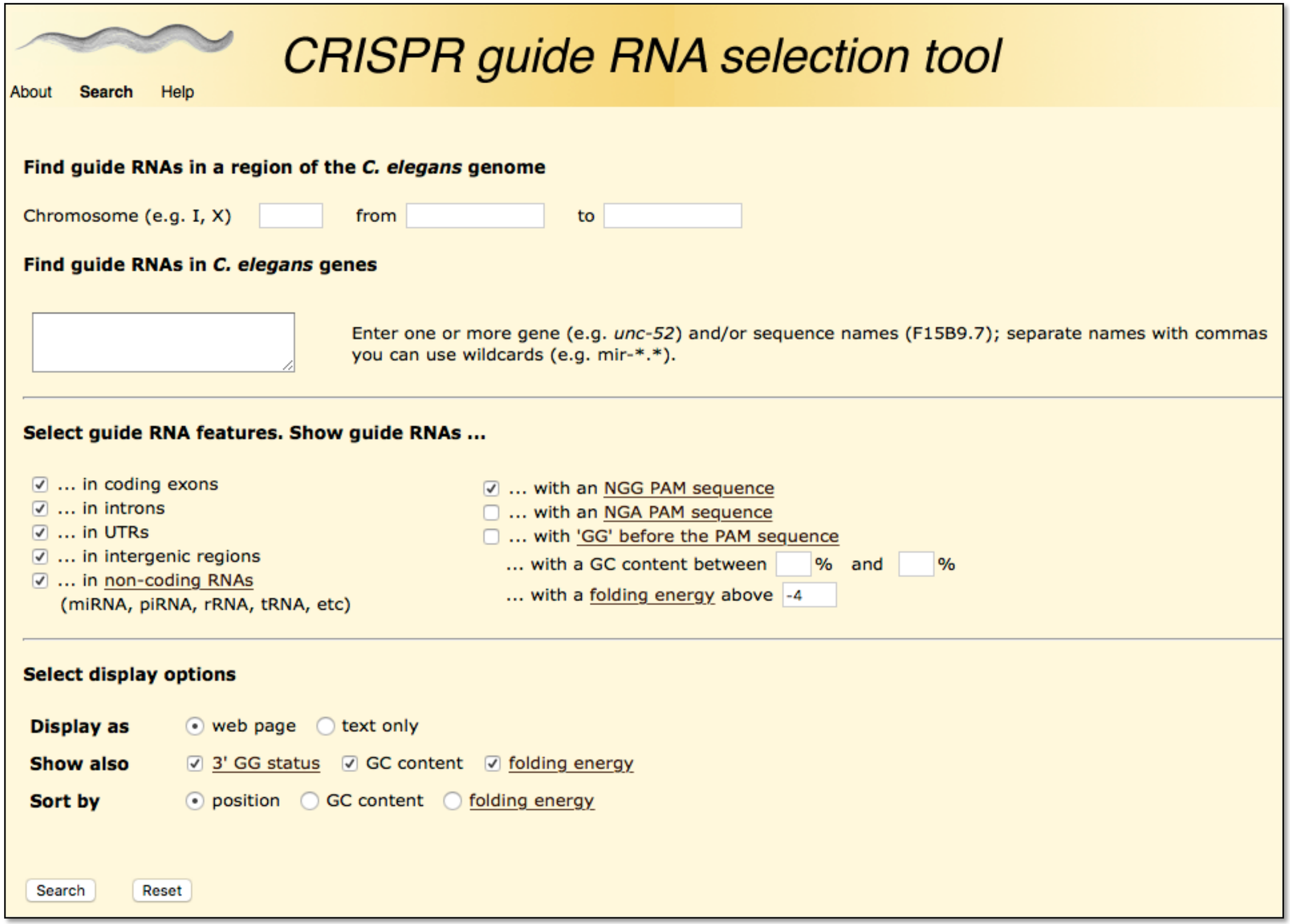
Site: genome.sfu.ca/crispr

All possible NGG and NGA guides in database

Default Filters:

Sequence and annotation from WS250
GC content between 20% and 80%
No poly-T tracts 5bp or longer
Unique seed region (12 bp at 3’ end plus PAM)
Folding energy > -4

CRISPR sgRNA Selection Site



CRISPR sgRNA Selection Site

CRISPR guide RNA selection tool

About **Search** Help

Find guide RNAs in a region of the *C. elegans* genome

Chromosome (e.g. I, X) from to

Find guide RNAs in *C. elegans* genes

unc-112

Enter one or more gene (e.g. *unc-52*) and/or sequence names (F15B9.7); separate names with commas you can use wildcards (e.g. mir-*.*).

Select guide RNA features. Show guide RNAs ...

- [x] ... in coding exons
- [ ] ... in introns
- [ ] ... in UTRs
- [ ] ... in intergenic regions
- [ ] ... in non-coding RNAs (miRNA, piRNA, rRNA, tRNA, etc)

- [x] ... with an NGG PAM sequence
- [ ] ... with an NGA PAM sequence
- [x] ... with 'GG' before the PAM sequence

... with a GC content between % and %

... with a folding energy above -4

Select display options

Display as (•) web page ( ) text only

Show also [x] 3' GG status [x] GC content

Sort by (•) position ( ) GC content

Search Reset

Hot links to WormBase.

CRISPR guide RNA selection tool

About **Search** Help

5 guide RNAs found

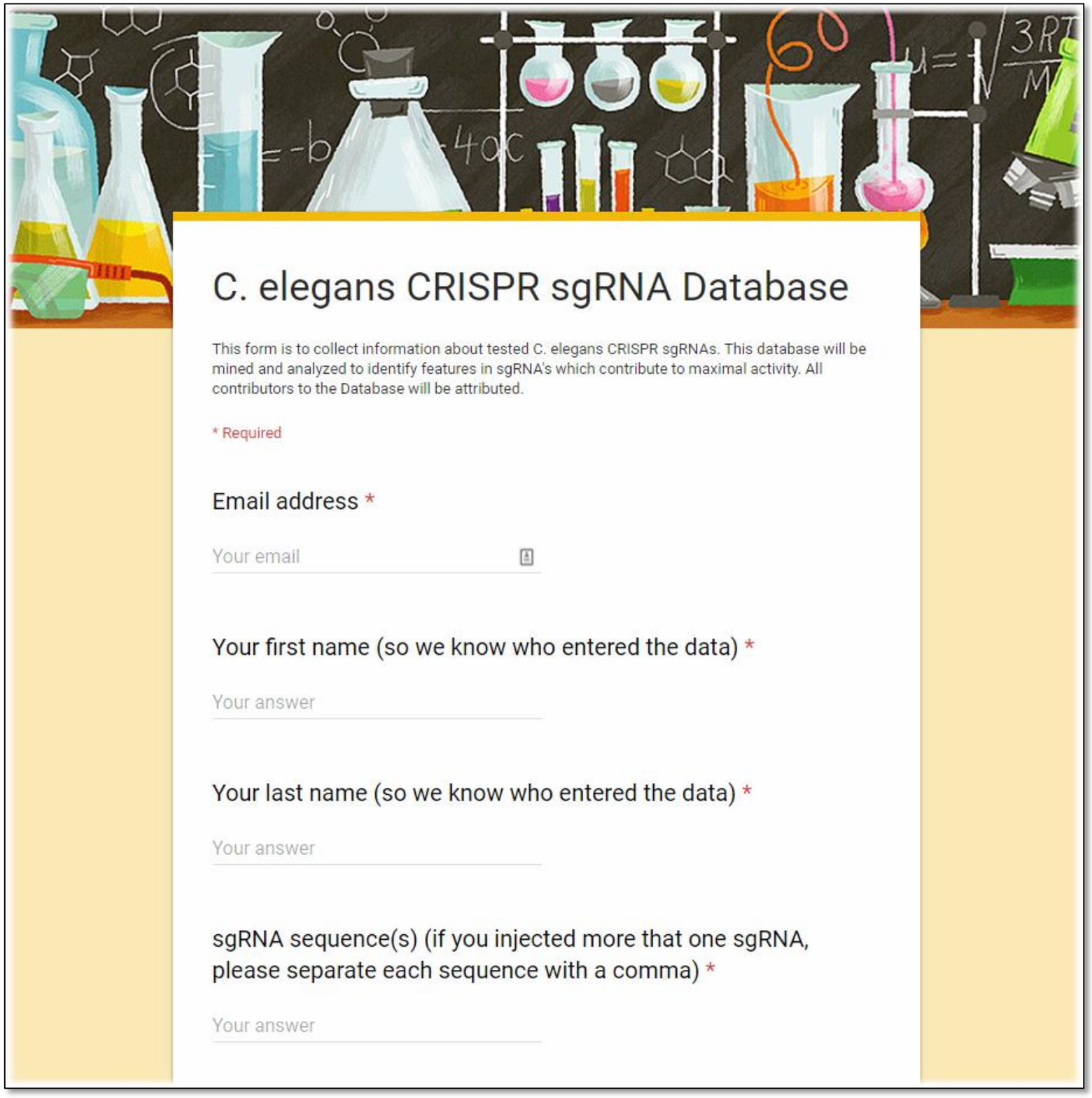
Seq.name	Gene	Feature	Chr	Strand	Cut site position	Guide Sequence	3' GG status	PAM	GC content	folding energy
C47E8.7	unc-112	coding_exon	V	-	14692245	GTCGTTCACGAGTTCATCGG	yes	NGG	55	-3.2
C47E8.7	unc-112	coding_exon	V	-	14692967	GAGAAAGTCTGAAGACATGG	yes	NGG	45	-2.8
C47E8.7	unc-112	coding_exon	V	-	14693426	TGATTCGTCATATCAACAGG	yes	NGG	40	-2.6
C47E8.7	unc-112	coding_exon	V	-	14693490	CCGAGCATCAATATGCCAGG	yes	NGG	55	-3.9
C47E8.7	unc-112	coding_exon	V	-	14695037	GGTCTGATCATGCTCTGTGG	yes	NGG	55	-1.4

Notes: gene names are from Wormbase release WS250.

CRISPR sgRNA Selection Site

IGV display of all available guides in *flp-8* region

Guide Efficacy Survey Site



C. elegans CRISPR sgRNA Database

This form is to collect information about tested C. elegans CRISPR sgRNAs. This database will be mined and analyzed to identify features in sgRNA's which contribute to maximal activity. All contributors to the Database will be attributed.

* Required

Email address *

Your email

Your first name (so we know who entered the data) *

Your answer

Your last name (so we know who entered the data) *

Your answer

sgRNA sequence(s) (if you injected more that one sgRNA, please separate each sequence with a comma) *

Your answer

Guide Efficacy Survey Site

sgRNA target gene name *

Your answer

Did you use the following website to design this sgRNA:
http://genome.sfu.ca/crispr/search.html *

- Yes
- No
- Other: _____

Did this sgRNA cut (i.e., were you able to obtain your desired NHEJ- or HR-containing strain)? *

- Yes
- No

How many worms were injected? Please input a number. *

Your answer

If the sgRNA cut, how many desired lines/strains were obtained? Please input a number.

Your answer

Guide Efficacy Survey Site

Please rate the experience level of injector: *

	1	2	3	
Novice	○	○	○	Experienced

sgRNA was injected as *

- ○ DNA
- ○ RNA

If sgRNA was injected as DNA, what promoter was used?

- ○ R07E5.16 U6 promoter (Dickinson et al. 2013)
- ○ K09B11.12 U6 promoter (Friedland et al. 2013)
- ○ Other: _____

Cas9 was injected as: *

- ○ circular plasmid DNA
- ○ mRNA
- ○ protein
- ○ Other: _____

Guide Efficacy Survey Site

Screening method used to isolate CRISPR'ed worms *

- ◯ PCR and/or DNA sequencing
- ◯ Co-CRISPR
- ◯ Positive selectable markers
- ◯ Other: _____

Desired repair mechanism from CRISPR gene editing for this guide *

- ◯ NHEJ
- ◯ HR
- ◯ Other: _____

Any other comments you wish to add about your experience with this sgRNA

Your answer

☐ I'm not a robot

reCAPTCHA
Privacy - Terms

SUBMIT

Never submit passwords through Google Forms.

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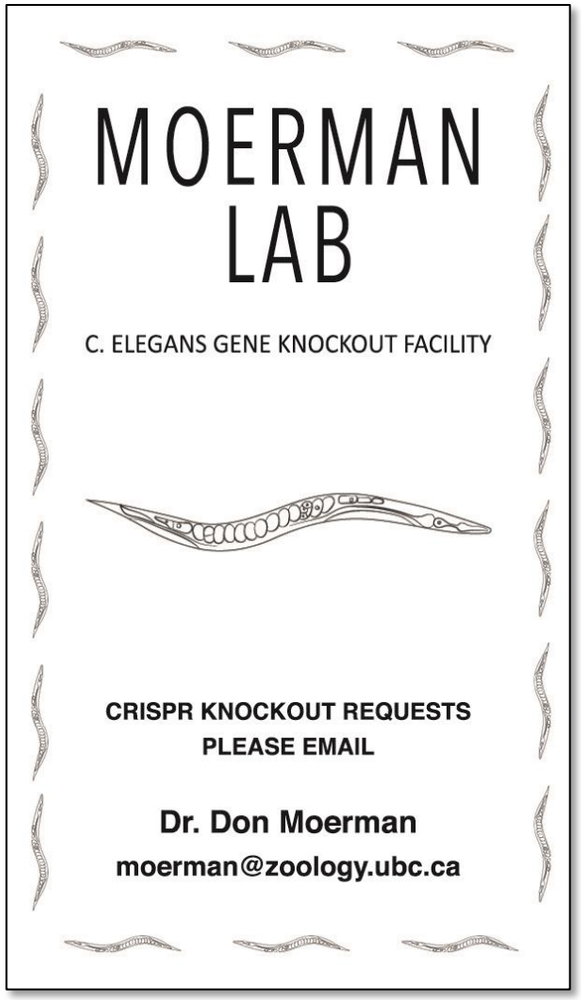

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http://genome.sfu.ca/crispr/help.html

About Search **Help**

CRISPR guide RNA selection tool

Basic questions

What is this database all about?

See the About page for details on CRISPR/Cas9 technology and how guide RNAs were identified and selected for this database.

What is an"*NGG PAM sequence*" ?

This is the protospacer adjacent motif (PAM) immediately following the crRNA target sequence in the genomic DNA. Cas9 protein variants all require a unique PAM site for binding and nuclease activity. NGG is the PAM site for Cas9 from Streptococcus pyrogenes.

What is an"*NGA PAM sequence*" ?

This PAM site is recognized by the VQR variant of Cas9 from Streptococcus pyrogenes (Kleistiver et al, Nature. 2015).

What is "*folding energy*" ?

The folding energy is the result of a minimum free energy calculation in kcal/mol for each guide in the database. Users should avoid selecting guides with large negative values as they are more likely to form stable secondary structures which could reduce efficiency.

What is "*GG before the PAM sequence*" or "*3' GG status*" ?

Farboud and Meyer (Genetics, 2015) found there was increased efficiency of Cas9 cutting if the 20 base pair protospacer (crRNA) ended in GG immediately before the PAM site.

What does "web page" versus "text only" mean?

Use "web page", if you want to view the search results in your web browser. Use "text only" for a tab-delimited text-based output in a browser window that can be copied and further processed locally. The sequence names in the html output table are hyperlinks to the corresponding gene page in Wormbase. The "cut site position" is a hyperlink to the genome browser view of the location in Wormbase.

Frequently asked questions

Why are there no guide RNAs in my gene of interest?

- make sure you use the correct type of identifier (gene name or sequence name, see Wormbase for details)
- make sure you don't use CGC gene names that are not in Wormbase release WB250
- make sure your search criteria are consistent (e.g. check 'RNA' under features, if your gene encodes a non-coding RNA

Why are there no guide RNAs in my chromosomal interval?

- make sure you haven't entered anything in the gene search field